Washington State University

CLOSEOUT REPORT
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Title: Calcium Messenger System in Gravitropic Response in Plants

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SUMMARY OF WORK COMPLETED

Calcium and calmodulin are known to play a central role in gravity signal transduction in plants. Calmodulin, upon binding of calcium, interacts with a number of proteins called calmodulin-binding proteins (CBPs) that play a key role in cellular regulation. To identify proteins that interact with calcium and calmodulin, a corn root tip cDNA expression library was screened with ^{35}S-labeled calmodulin to isolate cDNAs that code for CBPs. Several positive clones that bind to ^{35}S-calmodulin were isolated. Two CBP clones (CBP-1 and CBP-5) that bind to ^{35}S-labeled calmodulin as well as biotinylated calmodulin were sequenced and characterized. Comparison of the deduced amino acid sequence of both the clones showed 100% conservation of the 34 amino acid stretch at their carboxy-terminal end. Whereas, less homology was observed in other regions of the amino acid sequence. The highly conserved 34 amino acid stretch contained a putative calmodulin-binding domain, a basic amphiphilic alpha helix. The nucleotide and deduced amino acid sequence of both the clones did not show homology with any of the sequences in the nucleic acid and protein databases. Northern analysis with both CBP-1 and CBP-5 clones indicated that the corresponding genes are expressed in different parts of the root although there was a difference in the extent of expression.

We have also investigated the presence of Ca^{2+}/calmodulin-dependent protein kinase in corn root tips using affinity purified antipeptide antibodies produced against the α subunit of rat brain Ca^{2+}/CaM-dependent protein kinase II (CaM KII) and specific substrates for CaM KII. Three different antipeptide antibodies raised against CaM KII recognized a 56 kDa protein in soluble protein fraction isolated from corn root tips. The molecular weight of the plant protein that crossreacts with CaM KII antibodies is similar to the mammalian CaM KII. To confirm that the 56 kDa cross-reacting protein from root tips was a Ca^{2+}/CaM-dependent protein kinase, in-gel phosphorylation studies were performed. Incubation of gels containing immunoprecipitate in the presence of ATP, Ca^{2+} and CaM showed phosphorylation of a 56 kDa band. No phosphorylation of the 56 kDa band was observed in the presence of ATP alone or ATP and Ca^{2+}. These results indicate that the

56 kDa protein is a Ca^{2+}/CaM-dependent protein kinase and it undergoes autophosphorylation. The cross-reacting 56 kDa protein is also phosphorylated under *in vivo* conditions. Soluble extract from corn roots phosphorylated synapsin I, a physiological substrate for mammalian CaM KII, and this phosphorylation of synapsin I was stimulated by Ca^{2+} and CaM. Phosphopeptide mapping revealed that the site of enhanced phosphorylation of synapsin I was located within the 30 kDa fragment that is the known site of phosphorylation by CaM KII. BB40, a specific peptide substrate corresponding to residues 281-291 of the α subunit of CaM KII, was used to detect the CaM KII kinase activity in plants. Corn root soluble extract phosphorylated BB40 in a Ca^{2+}/CaM-dependent manner. These results suggest that plants contain a Ca^{2+}/CaM-dependent kinase. The role of this kinase in gravity signal transduction is being investigated.